PHARMACOLOGY

#927

Comm.
Dr. Gardner
Dr. Jacobson
Dr. Sommers

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

Application for Research Grant

Date: JUL. 25 1973

(Use extra pages as needed)

1. Principal Investigator (give title and degrees):

David J. Wilson, Ph.D., Professor of Chemistry

William Schaffner, II, M.D., Assistant Professor of Medicine

2. Institution & address:

Department of Chemistry

Vanderbilt University

Nashville, Tennessee 37235

3. Department(s) where research will be done or collaboration provided:

Department of Chemistry

Department of Medicine

4. Short title of study:

Nicotine Levels in Human Milk

5. Proposed starting date: 1 October 1973

6. Estimated time to complete: 12 months

7. Brief description of specific research aims:

The objective of this work is to determine the extent to which nicotine occurs in the milk of smoking nursing mothers.

8. Brief statement of working hypothesis:

The working hypothesis of the study is that the effects on nursling rats of dosing the mothers with nicotine is due to transmittal of nicotine through the milk and that similar transmittal may be taking place in humans.

9. Details of experimental design and procedures (append extra pages as necessary)

It is well established that a number of drugs administered to nursing mothers are excreted in their milk; Catz and Giacoia cite some 84 references in their review on the subject.[1] It is also well established that smoking by pregnant women has a number of effects upon the fetus -- the newborn infants tend to be smaller than normal and there appears to be an increase in abortions and stillbirths.[2,3,4,5,6,7] The lethal dose of nicotine for an adult is roughly 60 mg; a smoker typically absorbs 2-3 mg of the alkaloid per cigaret. Nicotine is deactivated in the liver and excreted via the kidneys. In small doses it affects the cholinergic synapses, causes release of adrenaline, and shifts the brain's EEG toward an arousal pattern.[8] Although nicotine in human milk is mentioned in the literature from time to time,[9,10,11] there does not seem to be any appreciable amount of data available on the subject. A recent study on the effects on nursling rats of dosing the mother rats with small quantities of nicotine indicated that the drug (or possibly a toxic metabolite) was transmitted to the young rats with deleterious effect.[12] It is difficult to relate this study to possible effects on nursing human infants of smoking mothers, but its findings are thought-provoking.

We therefore propose to analyze approximately 50 samples of human milk for nicotine. Of these, a small (5-10) control group will be obtained from non-smokers, with the rest coming from light, moderate, and heavy smokers. About half of these samples are already available, left from a study on DDT levels which we recently completed; the remainder will be solicited from La Leche League, a women's organization concerned with breast feeding which was very helpful in providing samples and helpful suggestions for our DDT project and current work which we are doing on lead levels in human milk. A questionnaire will be used to obtain information about smoking and dietary habits, age of mother and infant, parity of mother, etc.

(see continuation page)

9. Details of experimental design and procedures (continued)

Nicotine will be determined in the samples by means of a modification of the gas chromatographic technique adapted by Burrows and coworkers[13] from a method due to Schievelbein and Grundke.[14] This method is capable of determining nicotine levels in the nanogram/ml range, and is much more sensitive than gas chromatographic methods for nicotine in urine.[15,16] The sample (10 ml) is made alkaline and steam-distilled, and the distillate is made alkaline and extracted with methylene chloride. This extract is cleaned up on an alumina column; nicotine is eluted with 1-1 methylene chloride-ethyl alcohol, and this solution is chromatographed (8% carbowax 20 M + 2% KOH on Chromosorb W, acid washed and treated with hexamethyl-disilazane) at 150°C, using a flame ionization detector.

BIBLIOGRAPHY

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2 The Health Consequences of Smoking, Publ. Health Serv. Publication No. 1696, Washington, D. C., U. S. Gov't. Printing Off., 1967.

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13 I. E. Burrows, P. J. Corp, G. C. Jackson and B. F. J. Page, Analyst (London) 96, 81 (1971).

14 H. Schievelbein and K. Grundke, Fresenius' Z. Analyt. Chem. 237, 1 (1968).

15 A. H. Beckett and E. J. Triggs, Nature 211, 1415 (1966).

16 N. L. McNiven, K. H. Raisiughani, S. Patashnik, and R. I. Dorfman, Nature 208, 788 (1965).

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

We have available a Varian 600-D gas chromatograph equipped with a flame ionization detector for use in this study. The University's Sigma 7 computer is more than adequate for our statistical needs, and we have programs currently in use which will take care of the data processing in the proposed project. An LKB-9000 combined mass spectrometer-gas chromatograph is available in the department for confirmation of compound identity. The science and medical libraries of the University are quite adequate for the needs of the project, and ample laboratory space is available.

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

12. Biographical sketches of investigator(s) and other professional personnel.

13. Publications.

CURRICULUM VITAE OF DAVID J. WILSON

We have available a Varian 600-D gas chromatograph equipped with a flame

EDUCATION:

B.S.	1952	Stanford University, Stanford, California
Ph.D.	1958	California Institute of Technology, Pasadena, California

SCIENTIFIC EXPERIENCE:

1. Stanford University (1952-53) - National Science Foundation Fellow in chemistry, research on the thermal decomposition of nitrogen pentoxide in the presence of nitric oxide, under Dr. H. S. Johnston.
2. Army Chemical Center, Maryland (1953-55) - Physical sciences assistant, Analytical Branch, Chemical Division, Chemical and Radiological Laboratories. Director; Mr. Sam Sass. Analytical research and routine analyses connected with organic phosphonates.
3. Stanford University (1955-56) - National Science Foundation Fellow in chemistry, research on the thermal decomposition of nitryl chloride, on the computation of pre-exponential factors in gas-phase reactions, and on the isotope effect in the oxidation of carbon monoxide by nitrogen dioxide. This work was done under the direction of Dr. H. S. Johnston.
4. California Institute of Technology (1956-57) - National Science Foundation Fellow in chemistry, research on the thermal decomposition of nitryl chloride and on temperature gradients in reaction cells, under Dr. H. S. Johnston.
5. University of Rochester (1957-69) - Instructor (1957-60), assistant professor (1960-63), associate professor (1963-67), and professor of chemistry, research on the theory of energy transfer processes in gas reactions, on the sensitized photodecomposition of nitryl chloride, in nuclear magnetic resonance, and in the quantum theory of inelastic scattering; undergraduate and graduate instruction in chemistry; section editor, Chemical Abstracts (1958-62); Alfred P. Sloan Fellow (1964-66). Visiting Senior Lecturer, University of Ife, Nigeria (1964-65).
6. Vanderbilt University (1969-Present) - Professor of chemistry; research in gas reactions and energy transfer processes in gases, investigation of pesticide and heavy metal residues, foam flotation methods, undergraduate and graduate instruction in chemistry.

PROFESSIONAL SOCIETIES:

1003542190

REDACTED

PUBLICATIONS OF DAVID J. WILSON

1. "Decomposition of Nitrogen Pentoxide in the Presence of Nitric Oxide. IV. Effect of Noble Gases,: David J. Wilson and Harold S. Johnston, J. Amer. Chem. Soc., 75, 5763 (1953).
2. "Theoretical Pre-exponential Factors for Hydrogen Atom Abstraction Reactions," David J. Wilson and Harold S. Johnston, J. Amer. Chem. Soc., 79, 29 (1957).
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4. "Temperature Gradients in Reactions Cells," David J. Wilson, J. Phys. Chem., 62, 653 (1958).
5. "Solution of Systems of Linear Equations in Analytical Chemistry," David J. Wilson, Anal. Chem., 30, 1578 (1958).
6. "A Comparison of Slater's Theory of Unimolecular Reactions with Experimental Data," Everett Thiele and David J. Wilson, Can. J. Chem., 37, 1035 (1959).
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8. "Some Consideration of Unimolecular Rate Theory," Frank P. Buff and David J. Wilson, J. Chem. Phys., 32, 677 (1960).
9. "Intramolecular Processes in Unimolecular Reactions," David J. Wilson, J. Phys. Chem., 64, 323 (1960).
10. "An Extension of Slater's High Pressure Unimolecular Rate Expression to Simultaneous Reaction Coordinates," Everett Thiele and David J. Wilson, J. Phys. Chem., 64. 473 (1960).
11. "Proton Magnetic Resonance Studies. I. Cyclophanes," David J. Wilson, Rodger Griffin, and Virgil Boekelheide, J. Amer. Chem. Soc., 82, 6302 (1960).
12. "The Pressure Dependence of Fluorescence Spectra," David J. Wilson, Barbara Noble, and Betty Lee, J. Chem. Phys., 34, 1392 (1961).
13. "Anharmonicity in Unimolecular Reaction," Everett Thiele and David J. Wilson, J. Chem. Phys., 35, 1256 (1961).
14. "Photochemical Decomposition of Nitryl Chloride," Abraham S. Dohner and David J. Wilson, J. Chem. Phys., 35, 1510 (1961).
15. "Intermolecular Energy Transfer in Gas Reactions," Narl Chow and David J. Wilson, J. Phys. Chem., 66, 342 (1962).
16. "Pressure Dependence of Fluorescence Spectra. II. Transient Effects," David J. Wilson, J. Chem. Phys., 36, 1293 (1962).

17. "Pressure Dependence of Fluorescence Spectra. III. Effect of Finite Pulse Length," Joseph W. Brauner and David J. Wilson, J. Chem. Phys., 36, 2547 (1962).

18. "Pressure Dependence of Fluorescence Spectra. IV. Effects of Vibrational Energy Transfer between Fluorescing Molecules," Robert C. Davis and David J. Wilson, J. Chem. Phys., 37, 848 (1962).

19. "Classical Unimolecular Rate Theory. Rotating Anharmonic Diatomic Molecules," Frank P. Buff and David J. Wilson, J. Amer. Chem. Soc., 84, 4063 (1962).

20. "Energy Transfer Processes in Gas Reactions," David J. Wilson, Bull. Soc., Chim. Belg., 71, 664 (1962).

21. "Anharmonic Effects in Unimolecular Rate Theory. Dynamics of a Rotating Anharmonic Triatomic Molecule," Narl Chow Hung and David J. Wilson, J. Chem. Phys., 38, 828 (1963).

22. "Photochemical Reactions in the Gas Phase and Slater's New Approach to Rate Theory," David J. Wilson, J. Chem. Phys., 38, 1098 (1963).

23. "Intramolecular Energy Transfer in Unimolecular Reactions. II. A Weakly-coupled-oscillators Model," Joseph W. Brauner and David J. Wilson, J. Chem. Phys., 67, 1134 (1963).

24. "Anharmonic Effects in Unimolecular Rate Theory. Vibrations and Collisions of Simple Polyatomic Systems," Robert J. Harter, Elliott B. Alterman, and David J. Wilson, J. Chem. Phys., 40, 2137 (1964).

25. "The Thermal Decomposition of Nitryl Chloride in Solution," David Beggs, Catherine Block, and David J. Wilson, J. Phys. Chem., 68, 1494 (1964).

26. "A Quantum Mechanical Formulation of a Strong Collision Theory of Unimolecular Reaction," David J. Wilson and Everett Thiele, J. Chem. Phys., 40, 3425 (1964).

27. "Classical Unimolecular Rate Theory. II. Effect of the Distribution of Initial Conditions," Roger Baetzold and David J. Wilson, J. Phys. Chem., 68, 3141 (1964).

28. "Thermodynamic Functions of More Oscillators," Roger W. Crecely and David J. Wilson, J. Chem. Phys., 41, 1564 (1964).

29. "Vibrational Energy Transfer in Gases. Atomic-Diatomic Molecule Collisions," Elliott B. Alterman and David J. Wilson, J. Chem. Phys., 42, 1957 (1965).

30. "Classical Unimolecular Rate Theory. III. Effect of Initial Conditions on Lifetime Distributions," Roger C. Baetzold and David J. Wilson, J. Chem. Phys., 43, 4299 (1965).

31. "Some Considerations of Unimolecular Rate Theory. II. Aspects of the General Theory," Frank P. Buff and David J. Wilson, J. Chem. Phys., 45, 1444 (1966).

32. "Pressure-dependent Transmission Coefficients. Isomerization of a Restricted Rotor," Eric Herbst and David J. Wilson, J. Chem. Phys., 45, 1442 (1966).

33. "The Quantum-Dynamics of Anharmonic Oscillators. I. Simple Examples," Elliott B. Alterman, Charlotte M. Tahk, and David J. Wilson, J. Chem. Phys., 44, 451 (1966).

34. "Quantum-Dynamics of Anharmonic Oscillators. II. Systems Having One and Two Degrees of Freedom," Roger C. Baetzold, Charlotte T. Tahk, and David J. Wilson, J. Chem. Phys., 45, 4209 (1966).

35. "Quantum-Dynamics of Anharmonic Oscillators. III. The Morse Oscillator," Paul F. Endres and David J. Wilson, J. Chem. Phys., 46, 4205 (1967).

36. "Application of the WKB Method to the Dynamics of Anharmonic Oscillators," R. Dubrow, D. Hatzenbuhler, W. Marx, E. Zahorian, and D. J. Wilson, J. Phys. Chem., 72, 2489 (1968).

37. "The Quantum Dynamics of Triatomic Molecules," William E. Smyser and D. J. Wilson, J. Chem. Phys., 50, 182 (1969).

38. "Vibrational Energy Transfer in Gases: Atom-Triatomic Molecule and Diatomic-Diatomic Molecule Collisions," Robert Dubrow and D. J. Wilson, J. Chem. Phys., 50, 1553 (1969).

39. "Quantum Transition Probabilities for Atom-Triatomic Molecule Collisions," John J. Grimaldi, Paul F. Endres, and David J. Wilson, J. Chem. Phys., 50, 1627 (1969).

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43. "Quantum Vibrational Transition Probabilities in Atom-Diatomic Molecule Collisions. III. Reactive Scattering," D. J. Wilson, J. Chem. Phys., 51, 5008 (1969).

44. "Pyrolysis of Ethylcyclobutane in the Gas Phase at High Pressures," J. Aspden, N. A. Khawaja, J. Reardon, and D. J. Wilson, J. Amer. Chem. Soc., 91, 7580 (1969).

45. "Exact Semiclassical Transition Probabilities for Collinear Collisions," D. J. Wilson and D. J. Locker, J. Chem. Phys., 52, 271 (1970).

46. "The Quantum Dynamics of Dissociating Oscillators," C. T. Tahk, Y. R. L. Park, and D. J. Wilson, J. Chem. Phys., 53, 786 (1970).

47. "Quantum Vibrational Transition Probabilities in Diatomic-Diatomic Molecule Collisions," D. J. Wilson, J. Chem. Phys., 53, 2075 (1970).

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50. "Quantum Vibrational Transition Probabilities in Atom-Diatomic Molecule Collisions. V. Effects of Mass and Well Depth," D. J. Wilson, J. Chem. Phys., 54, 540 (1971).

51. "Quantum Vibrational Transition Probabilities in Atom-Diatomic Molecule Collisions. A Tractable Three-Dimensional Model," D. J. Wilson and D. J. Locker, J. Chem. Phys., 57, 5393 (1972).

52. "DDT Concentrations in Human Milk," D. J. Wilson, D. J. Locker, C. A. Ritzen, and J. T. Watson, Amer. J. Diseases Children, 125, 814 (1973).

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54. "Hexachlorophene Levels in Human Milk," Robert West and David J. Wilson, manuscript in preparation.

55. "Lead Levels in Human Milk," H. Kenneth Dillon and David J. Wilson, manuscript in preparation.

56. "Non-Ideal Line Shapes in Gas Chromatography," Sheng-Da Huang, John W. Wilson, and David J. Wilson, manuscript in preparation.

CURRICULUM VITAE OF WILLIAM SCHAFFNER, II

NAME William Schaffner, II

DATE OF BIRTH REDACTED

MARRIED REDACTED

CHILDREN

PRESENT ADDRESS

PRESENT POSITION Assistant Professor of Medicine, Director, Clinical Bacteriology Laboratory, Hospital Epidemiologist

DEGREES			
	B.S.	1957	Yale University
	M.D.	1962	Cornell University Medical College

INTERNSHIP, RESIDENCIES, FELLOWSHIPS, AND MILITARY SERVICE:

1.	Intern in Medicine, Vanderbilt University Hospital	1962-63
2.	Assistant Resident in Medicine, Vanderbilt University Hospital	1963-64
3.	USPHS Postdoctoral Fellow in Infectious Disease, Vanderbilt University School of Medicine	1964-66
4.	Epidemic Intelligence Service Officer of the National Communicable Disease Center, USPHS, Assigned to Rhode Island Department of Health; was Acting Chief, Division of Epidemiology	1966-68
5.	Chief Medical Resident, Vanderbilt University Hospital	1968-69

ACADEMIC AWARDS, etc.:

1.	Ford Foundation Scholar, Yale University	1953-57
2.	Fulbright Fellowship to Albert-Ludwigs University, Freiberg, Germany	1957-58
3.	New York City Health Research Council Summer Fellowship	1960
4.	L.S.U. Student Fellow in Inter American Program in Tropical Medicine, Guatemala (2 months)	1962
5.	USPHS Postdoctoral Fellowship	1964-66
6.	Fellow, Fifth International Teaching Seminar on Cardiovascular Epidemiology, Singapore	1972

William Schaffner, M. D. - Curriculum Vitae

MEMBERSHIP IN PROFESSIONAL SOCIETIES:

1.
2.
3. REDACTED
4.
5. REDACTED
6. REDACTED
7.

LICENSE:

Tennessee REDACTED

BOARD CERTIFICATION:

1. Diplomate of the National Board of Medical Examiners
2. Diplomate of the American Board of Internal Medicine
3. Diplomate in the subspecialty of Infectious Diseases (American Board of Internal Medicine)

PUBLICATIONS OF WILLIAM SCHAFFNER, M.D.

1. "The Diarrhea of Travelers. V. Prophylaxis with Phthalysulfathiazole and Neomycin Sulfate," B. H. Kean, W. Schaffner, R. W. Brennan, and S. R. Waters, J. A. M. A., 180, 367 (1962).
2. "The Diarrhea of Travelers. IV. Viral Studies of Visiting Students in Mexico with Further Bacteriologic and Parasitologic Observations," M. A. Rosenbluth, W. Schaffner, and B. H. Kean, Am. J. Trop. Med., 12, 239 (1963).
3. "Thrombocytopenic Rocky Mounty Spotted Fever: Case Study of a Husband and Wife," W. Schaffner, A. C. McLeod, and M. G. Koenig, Arch. Int. Med., 116, 857 (1965).
4. "Fatal Pneumonia Due to a Tetracycline-resistant Pneumococcus," W. Schaffner, W. M. Schreiber, and M. G. Koenig, New Eng. J. Med., 274, 451 (1966).
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7. "Lysostaphin: An Enzymatic Approach to Staphylococcal Disease. I. In Vitro Studies," W. Schaffner, M. A. Melly, J. H. Hash, and M. G. Koenig, Yale J. Biol. Med., 39, 215 (1967).
8. "Lysostaphin: An Enzymatic Approach to Staphylococcal Disease. II. In Vivo Studies," W. Schaffner, M. A. Melly, and M. G. Koenig, Yale J. Biol. Med., 39, 230 (1967).
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11. "Infection Following Cardiovascular Surgery: Clinical Study Including Examination of Antimicrobial Prophylaxis," J. S. Goodman, W. Schaffner, H. A. Collins, E. J. Battersby, and M. G. Koenig, New Eng. J. Med., 278, 117 (1968).
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13. "The Clinical Epidemiology of Sporadic Measles in a Highly Immunized Population," W. Schaffner, A.E. Schluederberg, and E. B. Byrne, New Eng. J. Med., 279, 783 (1968).

14. "Rubella Antibodies in Rhode Island Women of Child-bearing Age," E. B. Byrne, R. L. Petrelli, W. Schaffner, and M. C. Hinchliffe, Pub. Health Rep., 84, 139 (1969).

15. "A Smallpox Vaccination Campaign for Hospital Personnel in Rhode Island," W. Schaffner and R. M. Adair, Pub. Health Rep., 84, 425 (1969).

16. "Hospital Outbreak with Group-A Streptococci Traced to an Asymptomatic Anal Carrier," W. Schaffner, L. B. Lefkowitz, J. S. Goodman, and M. G. Koenig, New Eng. J. Med., 280, 1224 (1969).

17. "Botulism," Chapter 26, Vol. III of Tice's Practice of Medicine, Hoeber Medical Division, Harper and Row Publishers, Inc., Hagerstown, Md., 1970 (W. Schaffner and M. G. Koenig).

18. "Infant Immunization Surveillance: Cost Versus Effect. A Prospective Controlled Evaluation of a Large Scale Program in Rhode Island," E. B. Bryne, W. Schaffner, E. Dini, and G. W. Case, J. A. M. A., 212, 770 (1970).

19. "Two Syndromes Following Rubella Immunization. Clinical Observations and Epidemiological Studies," A. W. Kilroy, W. Schaffner, W. F. Fleet, L. B. Lefkowitz, D. T. Karzon, and G. M. Fenichel, J. A. M. A., 214, 2287 (1970).

20. "Severe Influenza Virus Pneumonia in the Pandemic of 1968-1969," R. F. Burk, W. Schaffner, and M. G. Koenig, Arch. Int. Med., 127, 1122 (1971).

21. "Superinfection in Lymphoreticular Diseases." Annual Review of Medicine, Vol. 22. Annual Reviews, Inc., Palo Alto, Calif., 1971, pp 25-38 (Z. A. McGee, W. Schaffner, and M. G. Koenig).

22. "Innovation in Communicable Disease Reporting," W. Schaffner, H. D. Scott, B. J. Rosenstein, and E. B. Byrne, HSMHA Health Rep., 86, 431 (1971).

23. "The Use of Marginal-punched Data Cards in Surveillance of Hospital-acquired Infection," L. B. Lefkowitz, G. B. Lavely, and W. Schaffner, HSMHA Health Rep., 86, 953 (1971).

24. "Measles Eradication: The Impossible Dream?" W. Schaffner, Proceedings of the Eighth Immunization Conference, Center for Disease Control, USPHS, HSHMA, DHEW, Atlanta, Ga., pp 15-16, 1971.

25. "Efficacy and Safety of Topical Lysostaphin Treatment of Persistent Nasal Carriage of S. aureus., K. E. Quickel, R. Selden, J. R. Caldwell, N. S. Nora, and W. Schaffner, Appl. Micro., 22, 446 (1971).

26. "Exposure of Susceptible Teachers to Rubella Vaccinees," W. F. Fleet, W. Schaffner, L. B. Lefkowitz, G. D. Murphy, and D. T. Karzon, A,. J. Dis. Child., 123, 28 (1972).

27. "Botulism," Chapter in The Science and Practice of Clinical Medicine, edited by J. Dietschy, et al, Grune and Stratton, Inc., New York (in press) (W. Schaffner).

28. "The Postoperative Detection of Salmonella typhi: An Unexpected Hospital Infection Hazard," G. Reisig and W. Schaffner, Arch. Surg., 104, 349 (1972).

29. "Microbiological Safety of Solutions and Delivery to the Patient: Problems in Preparation and Handling," W. Schaffner, In Proceedings of the Symposium on Total Parenteral Nutrition, Nashville, Tenn., Jan. 17-19, 1972. Food Science Committee, Council on Foods and Nutrition of the American Medical Association, pp 126-131.

30. "Topics in Infectious Diseases: Current Antibiotic Sensitivities of Gram-negative Bacteria," W. Schaffner, H. B. Ratner, and M. G. Koenig, J. Tenn. Med. Association, 65, 615 (1972).

31. "Rocky Mountain Spotted Fever. In Current Therapy - 1973," W. Schaffner, edited by H. F. Conn, W. B. Saunders Co., Philadelphia, Pa., (in press).

32. "Topics in Infectious Diseases: Increasing Resistance of Shigellae to Antibiotics," R. L. Harbin, H. B. Ratner, and W. Schaffner, J. Tenn. Med. Association, 65, 999 (1972).

33. "Sepsis Due to Contaminated Intravenous Fluids: Epidemiologic, Clinical, and Laboratory Observations in One Hospital," S. K. Felts, W. Schaffner, M. A. Melly, and M. G. Koenig, Ann. Int. Med., 77, 881 (1972).

34. "Septicemia and Total Parenteral Nutrition: Distinguishing Catheter-related from Other Septic Episodes," J. D. Dillon, W. Schaffner, C. W. VanWay, and H. C. Meng, J. A. M. A., (in press).

35. "DDT Levels in Human Milk," D. J. Wilson, D. J. Locker, C. A. Ritzen, J. T. Watson, and W. Schaffner, Am. J. Dis. Child., 125, 814 (1973).

36. "Infection of an Avulsed Papillary Muscle Tip Simulating Bacterial Endocarditis," T. K. Satterwhite, Z. A. McGee, W. Schaffner, G. C. Friesinger, M. Mishu, and R. D. Collins, Am. Heart J. (in press).

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38. "Hospital-acquired Infections," Chapter in Infectious Diseases in Obstetrics and Gynecology, edited by G. R. G. Monif, Hoeber Medical Division, Harper and Row Publishers, Inc., Hagerstown, Md., (in press) (W. Schaffner).

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ABSTRACTS:

1. "Lysostaphin: An Enzymatic Approach to the Therapy of Experimental Staphylococcal Infections," W. Schaffner, M. A. Melly, and M. G. Koenig, Clin. Res., 14, 343 (1966).
2. "An Outbreak of Sepsis Due to Contaminated Intravenous Fluid: Clinical, Epidemiological and Laboratory Observations," W. Schaffner, S. K. Felts, M. A. Melly, and M. G. Koenig, Ann. Int. Med., 76, 872 (1972).

14. First year budget.

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
1. David J. Wilson	20	REDACTED
2. William Schaffner	10	
Technical		
1. Bruce Ferguson	100	REDACTED
	Sub Total for A	REDACTED
B. Consumable supplies (by major categories)		
1. Chromatographic supplies and service		350
2. Chemicals		460
3. Mass spectrometer analyses		40
4. Glassware		200
5. Computer time		53
	Sub-Total for B	1,103
C. Other expenses (itemize)		
None		
	Sub-Total for C	0
	Running Total of A + B + C	REDACTED
D. Permanent equipment (itemize)		
None		
	Sub-Total for D	0
E Indirect costs (15% of A+B+C)	E	1,170
15 Estimated future requirements	Total request	REDACTED

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip	Indirect Costs	Total
Year 2	Not applicable					
Year 3	Not applicable					

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
	None		

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Lead and Cadmium Levels in Human Milk and Human Deciduous Teeth	(submitted to NSF, EPA)	$37,222	1 Jan. '74 - 30 Dec. '75

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

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DDT Concentrations in Human Milk

David J. Wilson, PhD, David J. Locker, PhD; Charles A. Ritzen;
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Human milk from seven US cities was analyzed for total DDT (DDT plus DDE) content. The mean of 138 samples was 0.17 ppm (range, <0.02 to 0.83 ppm) which is in excess of the World Health Organization's recommended maximum concentration in cow's milk (0.05 ppm.)

Use of commercial exterminators was associated with lower DDT levels than was personal home use of pesticides; donors using butter had lower concentrations than those using margarine. DDT levels diminished with increasing maternal age and milk obtained after nursing contained significantly more DDT than milk obtained at the start of nursing.

While no adverse effects to infants due to DDT in human milk has been documented, systematic monitoring of DDT and other environmental pollutants in man is needed.

Concern has been expressed in both the scientific literature[1,2] and the lay press[3] over the concentrations of DDT and its metabolites in human milk. This has resulted in some worry to women breast-feeding or planning to breast-feed infants.

The magnitude of public discourse has been somewhat disproportionate to the extent of the data. The number of pesticide residue determinations in human milk is small and the colorimetric methods employed in earlier work are open to some question.[4]

Only eight articles have appeared in the English language literature over the 27 years, 1945 to 1972—a period of great change in the extent of pesticide use. As reviewed by Ritcey et al,[5] seven additional studies have been published in the USSR and 12 in European countries. At present, the data are not sufficient to delineate geographic, racial, socioeconomic, and other possible variations in DDT concentrations in human milk. Preliminary data bearing on some of these questions are presented here.

Materials and Methods

Samples of human milk were obtained from white, urban, middle-class donors residing in several towns on Long Island and in Rochester, NY; Chicago; Lexington, Ky; Nashville and Memphis, Tenn; and Los Angeles. Samples were obtained during the period from June 1970 through October 1971. Donors also completed a brief questionnaire regarding their exposure to pesticides, food habits, and weight gain or loss. Samples were kept frozen in polyethylene bags or in glass bottles until analysis. Chlorinated hydrocarbon pesticides were extracted from these samples by the method described by Schafer et al.[6] Ten milliliters of the milk was saponified with potassium hydroxide solution (25% solution of potassium hydroxide) and then extracted with 10 ml of hexane. The hexane extract was then sealed in a glass ampule until analysis. This method of sample preparation quantitatively converts DDT to DDE and results will be expressed as concentrations of total DDT.

Quantification of DDE was achieved with a gas chromatograph equipped with an electron capture detector, field-emission type, or with another gas chromatograph also equipped with a radioative nickel electron capture detector (^{63}Ni). The field-emission electron capture detector (ECD) was calibrated with standard aliquots (2 to 10 ng) of DDE (Varian Associates Nanogen Pesticide Standards) in benzene which also established the linear range of this ECD. Each analysis of 10μl to 20μl portions of the hexane extracts was followed by an injection of the analytical standard (DDE) to compensate for the drift of the field-emission ECD. The ^{63}Ni ECD was calibrated with several aliquots of the analytical standard (0.05 to 0.20 ng DDE); a standard was also injected after each sample analysis to insure reliability. Samples which indicated high levels of DDE on first analysis were diluted 1:10 so that a 1μl to 2μl injection would deliver a quantity of DDE known to be in the linear range of detection to the ^{63}Ni ECD.

Sample analyses were equally well accomplished on a 2 meter × 3 mm column of 10% DC-200 on Anakrom ABS 80/90 mesh support at 197 C (also used at 210 C) and a 2 meter × 3 mm column of 3% Dex 300 on Chromosorb G-HP 80/100 diatomite mesh support at 215 C. Two other diatomite support columns were used to confirm the identity of DDE in selected samples: a 2 meter × 4 mm column of mixed 5% QF-1 and 5% SE-30 on Chromosorb W60/80 mesh (acid washed) and a 2 meter × 4 mm column of 25% SF-96 on Chromosorb W60/80 mesh (acid washed).

Blank analyses were run on distilled water samples to establish that reagents were not introducing spurious results. Preliminary studies using commercial cow's milk demonstrated that the plastic bags used for storage did not contaminate the samples. Analyses of fresh milk and milk which had been stored frozen in plastic bags for two months showed no discernible differences.

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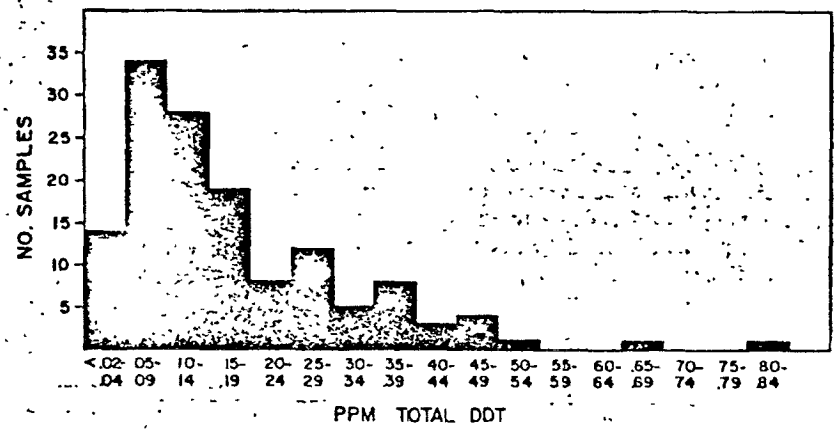

Frequency distribution of human milk samples by concentration of total DDT.

Table 1.—Total DDT Concentrations in Human Milk by City of Origin

City	No. Samples	Mean, ppm	Standard Deviation	P*
Long Island	14	0.100	0.10	.01
Rochester	20	0.17	0.13	NS†
Chicago	19	0.18	0.10	NS
Lexington	27	0.22	0.17	NS
Nashville	34	0.17	0.15	NS
Memphis	6	0.15	0.08	NS
Los Angeles	18	0.18	0.12	NS

* Probabilities of the difference between the corresponding mean and the mean of the total population being due to chance.
† Not significant.

Table 2.—Home Pesticide Use, Exterminator Use, and DDT in Human Milk

	No. Samples	Mean, ppm	Standard Deviation
Home Use			
Does not use pesticides	81	0.17	0.14
Uses pesticides	52	0.18	0.14
Exterminator Use			
No exterminators	107	0.18	0.15
Uses exterminators	30	0.14	0.10

Results

A total of 138 samples of human milk from 101 donors was analyzed. The mean total DDT concentration was 0.17 ppm with a standard deviation of 0.14 ppm. The range of concentrations was from less than 0.02 ppm to 0.83 ppm (Figure). Only four specimens had undetectable concentrations of total DDT (< 0.02 ppm) and three had concentrations in excess of 0.50 ppm.

The results are presented according to geographic area in Table 1. The mean concentration of the samples from the Long Island communities (0.10 ppm) is significantly lower than those from the other cities ($P = .01$). The mean of the Lexington samples exceeded that from the other areas, but this difference did not achieve statistical significance.

Of particular interest was the relationship of total DDT concentrations to prior reported exposure to pesticides (Table 2). Of the women, 39% had used pesticides in their homes or gardens, but there was not a statistically significant difference in the total DDT concentrations in the milk from the two groups. However, when those women employing exterminators were compared with those who used pesticides on their own, it was found that exterminator use was associated with lower concentrations of total DDT in the milk ($P = .05$). Frequent pesticide exposures at some time in the past, usually associated with agricultural activities, were reported by 26 women. The concentrations of total DDT in their milk did not differ significantly from those who were not so exposed.

Regarding diet, no significant correlations could be found between total DDT concentrations in milk and

Table 3.—DDT in Human Milk and Use of Butter or Margarine

	No. Samples	Mean, ppm	Standard Deviation
Butter	31	0.14	0.10
Margarine	40	0.20	0.16

Table 4.—Total DDT Concentrations (ppm) in Matched Fore-Milk and Hind-Milk Samples

Fore-Milk	Hind-Milk	Difference
0.09	0.13	+0.04
0.04	0.12	+0.08
0.06	0.16	+0.10
0.13	0.26	+0.13
0.03	0.06	+0.03
0.04	0.08	+0.04
0.23	0.35	+0.12
0.10	0.21	+0.11
0.05	0.17	+0.12
0.29	0.37	+0.08
0.11	0.12	+0.01
0.14	0.16	+0.02
0.68	0.45	−0.23
0.29	0.52	+0.23
0.11	0.35	+0.24
0.06	0.09	+0.03
0.06	0.46	+0.40
0.16	0.11	−0.05
0.13	0.15	+0.02
0.07	0.09	+0.02
0.16	0.04	−0.12
0.06	0.18	+0.12
0.37	0.48	+0.11
0.03	0.24	+0.21

the number of days per week the donor ate meat or fish. However, when those women who used butter and margarine were compared (Table 3), those using margarine had a higher concentration of total DDT in their milk than did those who used butter ($P < .04$).

While there was no correlation with the infant's age at the time the milk sample was donated, there was a negative correlation between the mother's age and the total DDT concentration in her milk, ie, the older the mother, the lower the concentration tended to be.

Twenty-four matched pairs of milk samples were obtained. Milk from a full breast (fore milk) was compared with milk from a nearly empty breast (hind milk) from the same donor at the same feeding. The two sets of samples showed a striking difference (Table 4): the total DDT concentration was significantly higher in the hind milk ($P < .01$).

We sought to determine the dependence of milk DDT concentration upon the date of sample collection. These preliminary results suggest a seasonal dependence of total DDT concentration, with DDT concentration in the late summer ranging up to 60% (0.08 ppm) higher than DDT concentration in the latter part of the winter. Additional specimens obtained over an extended period of time will be needed to verify this seasonal periodicity.

Comment

Organochlorine pesticides are now universal pollutants; they can be detected in virtually all animal tissues, even those sampled in remote parts of the earth far from areas of large-scale pesticide use.[3] It is now accepted that low tissue concentrations of such pesticides may produce subtle injury to species of birds, fish, and other nontarget organisms.[3, 10] Concern over the potential effects of pesticide residues in man has led to extensive routine food-monitoring programs in this and other countries and upper limits of acceptable pesticide concentrations have been set for many food items including milk.

The monitoring of man has been considerably less extensive and less standardized; milk is a pertinent example. The World Health Organization (WHO) has set a practical residue limit for total DDT in cow's milk of 0.05 ppm.[11] The Food and Drug Administration uses this value as the maximum permissible concentration of total DDT in the regular monitoring of commercial cow's milk shipped in interstate commerce. The recent public controversy regarding breast milk apparently emanates from newspaper reports of the higher concentrations of DDT in human milk.[3] As has been noted, the data base supporting these reports is, in comparison to that for cow's milk, extremely small. Nevertheless, it and the results of the current study do support the general conclusion that human milk contains a higher concentration of total DDT than does cow's milk.

The higher concentration of DDT in human milk is not an unexpected finding. Pesticides tend to become more concentrated as one samples up a food chain[12]: that is, meat-eaters (including man) store more DDT in their tissues than do herbivores, such as cattle. Hence, human milk would be expected to contain more DDT than that from cows.

The mean concentrations of total DDT in all seven geographical areas sampled in this study were in excess of the WHO upper limit for cow's milk. This was also the case in the two recent publications that reported on samples from various parts of Pennsylvania[13] and Canada.[5]

The WHO maximum admissible daily intake of DDT is set at 0.01 mg/kg of body weight.[11] Thus, a 4 kg infant ought not ingest more than 0.04 mg of DDT per day. If an infant drinks approximately 650 ml of milk per day,[14] the milk must contain less than 0.06 ppm DDT if the WHO limit for cow's milk is not to be exceeded. The mean in our study was 0.17 ppm.

It is imperative to state at this point that we know of no demonstrated damage to breast-fed infants from DDT. Furthermore, the study of Hayes et al[15] indicates that adult men are not injured directly by prolonged high-level oral doses of DDT. Increased mortality among neonatal rats nursing very heavily DDT-treated mothers has been reported,[16] but the relevance of that study to man is conjectural. The absence of a direct connection with illness notwithstanding, it appears prudent to monitor human breast milk for pesticide content.

Both biological and environmental factors correlating with the concentration of DDT in breast milk were revealed in this study. Although the sample size is among the largest in the literature, it is still small and the results are regarded more as an impetus to further study than as a definitive investigation.

The lower DDT content in the milk from the Long Island communities suggests that there may be significant variation with geographical area. We have no explanation for this observation.

Pesticide exposure was a less clear correlate than expected. Large-scale exposure in the past was not associated with increased DDT content, nor was personal home use of pesticides. The employment of exterminators seemed to be protective, however: it is known that commercial operators rarely use organochlorine pesticides in dwellings. The suggestion of a seasonal influence on milk DDT concentration might be due to environmental factors such as seasonal changes in diet or changes in domestic or garden use of pesticides.

Eating margarine rather than butter was associated with higher DDT concentrations. While we are reluctant to imply a directly causal relationship with this single dietary item, it is of interest that margarine is made largely of cottonseed oil and that DDT has been used extensively in the cotton industry.[17] Some direct measurements of DDT residues in margarine and butter have been made during the period from 1964 to

1966, the Food and Drug Administration performed analyses on 53 samples of margarine, 18.9% contained DDT with an average concentration of 0.026 ppm. In 1967, 13% of 23 samples contained an average of 0.014 ppm DDT.[9]

In contrast, during 1970, 100 butter samples were analyzed, 23% contained DDT with an average concentration of 0.005 ppm. In 1971, 5% of 84 samples contained only trace amounts of DDT (J.R. Wessel, Food and Drug Administration, written communication, August 1972). These data support the hypothesis that consumers of margarine are more apt to be exposed to DDT residues than are those who eat butter. It is suggested that nursing mothers eat butter rather than margarine.

The biological variations in pesticide content of breast milk revealed in this study require that future sampling be more precisely defined than in the past. The very significant increase in total DDT content of hind milk as compared with fore milk was the most striking variation encountered. Also of importance was the diminishing DDT concentration with increasing age of the donor. This relationship is consistent with Kroger's observation[13] that DDT content appears to decrease with the increasing number of children nursed by the mother. Future work should specify fore or hind milk collections and include age- and parity-specific concentrations.

We wish to reiterate that we know of no demonstrated danger from DDT to breast-fed infants which would warrant giving up the known advantages of breast-feeding. Nevertheless, we do feel that DDT concentrations in human milk should be more widely investigated in different geographic, socioeconomic, and racial groups and that the various biological factors affecting DDT excretion in human milk receive attention.

Household uses of DDT were banned by the Department of Agriculture during the autumn of 1969. On June 14, 1972, the Administrator of the Environmental Protection Agency issued a ban on the general use of DDT which took effect on January 1, 1973.[19] In 1970, the last year for which data are are available, approximately 25 million pounds of DDT were used in the United States (B. Fielding, Environmental Protection Agency, oral communication, December 1972). Public health, quarantine uses, and a few minor crop uses of DDT are exempted from the general ban and are estimated to require a few thousand pounds of DDT annually. As of this writing, several industrial groups are suing to gain exemptions for certain other agricultural uses. The ban will go into effect while these suits are in progress, but should the exemptions be granted, usage is estimated to be about one half million pounds of DDT per year. The order also does not affect exports of DDT for use in other countries; therefore, significant amounts of this pesticide will continue to enter the earth's ecosystem.

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